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Short communication

## Potential artifact formation of dioxins in ball clay during supercritical fluid extraction

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### Abstract

Earlier surveys indicate that meat, fish and dairy products are the principal source of polychlorinated dibenzo-*p*-dioxin (PCDD) exposure in the diet. A recent finding by others of PCDDs in chickens that consumed a feed containing PCDD led to the finding of ball clay, an anti-caking agent, as the source. Supercritical fluid extraction (SFE) was studied as a means to isolate PCDDs from commercial ball clays using GC-electron capture detection ( $\mu$ ECD) as a means to screen for these contaminants. The finding of ng/g amounts and recoveries >100% in several samples of ball clay containing octachlorodibenzo-*p*-dioxin (OCDD) suggested that PCDD may form artifactually as a result of analysis. Studies on pentachlorophenol (PCP) fortified ball clay were carried out by SFE and soxhlet extraction and the results compared. The values obtained by SFE were considered more problematic. The results obtained from ball clay suggest that precautions need to be exercised when using SFE to analyze for dioxins in solid samples containing chlorophenols. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Supercritical fluid extraction; Ball clay; Artifacts; Dioxins

### 1. Introduction

Supercritical fluid extraction (SFE) has been proposed as a more selective substitute for the classical soxhlet extraction method for polychlorinated dibenzo-*p*-dioxins (PCDDs) [1]. However, the effectiveness of SFE in isolating PCDDs is dependent on both analyte solubility and on analyte-matrix binding. While the solubility of PCDDs in supercritical carbon dioxide (SC-CO<sub>2</sub>) does not appear to be a major problem, binding/desorption is, especially for solid or semi-solid samples such as soil, sediment, sludge and fly ash. Fly ash offers an example of the

extreme SFE conditions needed for extraction [2]. It has to be treated with acid before use of supercritical carbon dioxide with a benzene [3] or methanol [4] modifier, or by using a binary trifluoroacetic acid–toluene cosolvent system [5] or alternative extraction with supercritical N<sub>2</sub>O [3,4].

Temperature is another parameter that affects PCDD extractability. Langenfeld et al. [6] reported that extraction temperatures up to 200°C (9500 p.s.i.), without the use of a cosolvent, were needed to achieve rapid optimal recoveries of native PCDDs, especially for the highest congener, octachlorodibenzo-*p*-dioxin (OCDD). Maio et al. [7] used SC-CO<sub>2</sub> at 250°C (9400 p.s.i.) to effectively extract PCDDs from fly ash.

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The finding of ball clay as the source of PCDDs in several chicken samples, at the ng/g level, in mid-1997 [8,9] gave us the opportunity to evaluate the use of SFE on a different type of solid sample matrix. Ball clay, used as an anti-caking agent in soybean-containing meal, may in retrospect, have been the source of elevated PCDDs also found in several earlier studies [10–12]. We sought to isolate PCDDs by SFE in the hope of yielding extracts that would be sufficiently clean for reliable screening of these compounds by GC- $\mu$ ECD, without the need for expensive high resolution mass spectrometry (HRMS), which requires dedicated instruments and personnel [13]. With HRMS, extract cleanup is usually minimal, being sufficient only in removing compounds that directly interfere with the associated PCDD ions.

The question of artifactual formation is only briefly referred to in the literature, but there is the possibility for false positive PCDDs, e.g. in sediment [14]. Given the importance of PCDD analysis and the need to analyze these compounds at the pg/g level or lower, it is essential that the methods employed be free of this potential problem. With the use of high temperatures during SFE sample extraction, it is theoretically possible to form PCDDs, especially when  $\mu$ g/g or higher concentrations of the precursor chlorophenols are also present in the sample or sample extract. The purpose of this paper is to investigate the application of SFE to PCDD analysis in ball clays, to assess the efficacy and its potential for artifact formation, and compare it with soxhlet extraction.

## 2. Experimental

### 2.1. Ball clay samples, reagents and chemicals

Samples of ball clay from three different mines in Tennessee and Kentucky, and kaolin and bentonite were purchased from a local ceramic supply company (Del Val Potter's Supply Co., Wyndmoor, PA). The dioxin and furan standard toluene solutions (5  $\mu$ g/ml) in EPA method 8280 and pentachlorophenol (PCP) were purchased from AccuStandard (New Haven, CT). The standards contained 2,3,7,8-tetra-

chloro- (TCDD), 1,2,3,7,8-pentachloro- (PeCDD), 1,2,3,4,7,8-hexachloro- (HxCDD), 1,2,3,4,6,7,8-heptachloro- (HpCDD) and octachloro-dibenzo-*p*-dioxins (OCDD) and the corresponding polychlorinated dibenzofurans (PCDFs). Benzene (EM Science, Gibbstown, NJ), methanol (Burdick & Jackson, Muskegon, MI) and activated carbon (30 mesh, Nuchar WV-W, Covington, VA) were used without further purification. Prior to use, a 150 ml sample of benzene was concentrated under a stream of nitrogen and analyzed for PCDD contamination; none was detected. To remove other possible contaminants, the nitrogen gas was passed through activated carbon.

### 2.2. Supercritical fluid extraction

To prevent PCDD loss, the glassware, including concentrator tubes (10 ml, Kontes Glass, Vineland, NJ) were silanized prior to use with Sylon CT (Supelco, Bellefonte, PA). Twenty four ml extraction vessels (Keystone Scientific, Bellefonte, PA) were packed from top to bottom: six 13 mm diameter GF/D glass fiber frits (Whatman, GF/D), 5.0 g ball clay, two 13 mm frits, 2.0 g of activated carbon, then two 13 mm glass fiber frits. A two-vessel parallel extractor (Applied Separations, Allentown, PA) equipped with a  $-15^{\circ}\text{C}$  chiller bath was employed as described previously [15]. The oven was preheated to  $150^{\circ}\text{C}$ , the micrometering valve to  $120^{\circ}\text{C}$ , and the SFE vessels pressurized to reach final pressure of 680 bar (10,000 p.s.i.). A static period of 5 min was used and a dynamic flow-rate of 3 l  $\text{CO}_2$ /min set. The depressurized  $\text{CO}_2$  gas was allowed to pass through 6 ml SPE cartridges (1.0 g silica gel, United Chemical Technologies, Bristol, PA), pre-washed with benzene and methanol, for ca. 40 min for a total of 120 l  $\text{CO}_2$ . Following  $\text{CO}_2$  depressurization, 250  $\mu$ l of benzene was passed through the off-line tubing to transfer any residual extractants onto the SPE cartridge which was subsequently eluted with 5 ml benzene into a 10 ml concentration tube. The contents of each tube were slowly evaporated, the sidewall rinsed with benzene, taken to dryness by a stream of nitrogen, reconstituted with 25  $\mu$ l benzene, then transferred into a 100  $\mu$ l polypropylene microconical vial (Scientific Resources, Eatontown, NJ) for GC-ECD quantification.

### 2.3. Soxhlet extraction

A 500 ml round bottom flask containing 150 ml benzene was attached to a soxhlet extractor with a 40–60 ml glass extraction thimble containing 5.0 g ball clay mixed with glass beads. After extraction for 24 h (6 cycles/h), the extracts were concentrated in a similar fashion as indicated for SFE.

### 2.4. Gas chromatographic analysis

A Hewlett-Packard Model 6890 gas-chromatograph equipped with a  $\mu$ ECD™  $^{63}\text{Ni}$  detector and a nonpolar SGE BPX5 capillary column (60 m $\times$ 0.32 mm I.D.,  $df=0.25\ \mu\text{m}$ ) was used for analysis. The splitless capillary injector was held at 240°C and detector at 350°C. The helium (BOC grade 5.5) carrier gas linear velocity was adjusted to 29 cm/sec (2 ml/min) and held at the following oven ramp parameters: 75°C (2 min), 20°C/min to 275°C, held for 28 min for a total 40 min runtime. The nitrogen (BOC grade 0.2) makeup gas flow (10 ml/min) was used to optimize detector linearity and sensitivity for 2,3,7,8-TCDD. Gases were purified through an activated charcoal hydrocarbon trap and a high capacity gas purifier (Supelco Inc., Bellefonte, PA). A 2  $\mu\text{l}$  splitless injection of extract concentrate was made while the column was held at 75°C for 2 min. The injector purge (75 ml/min) was activated at 0.75 min, and shut of at 2 min from the start of the injection. The PCDD concentrations in the clay

samples were determined by comparing the area and height values obtained to those from authentic standards.

### 2.5. GC–MS–MS

Confirmatory analysis of PCP and PCDD/Fs was carried out by tandem mass spectrometry (MS–MS) with an ion trap Saturn 2000 instrument (Varian Inc., Walnut Creek, CA) on PCP, and OCDD in the ball clay SFE extracts. The parameters for confirmation were independently developed, but were similar to those reported by Hayward [16]. Direct sample introduction (DSI) with a chromatoprobe device (Varian) was used to increase the injection volume to 10  $\mu\text{l}$  of the SFE extracts in benzene. The injector program was: 85°C for 1 min (50:1 split), ramped to 275°C at 100°C/min (split off), then returned to 150°C after 10 min until 30 min (50:1 split). For GC, the separation used a 100% methylpolysiloxane (Rtx-1ms; Restek Inc, Bellefonte, PA) 25 m, 0.32  $\mu\text{m}$  I.D., 0.5  $\mu\text{m}$  film thickness analytical column (plus 5 m retention gap). The oven program was: 85°C for 3 min, ramped to 125°C at 25°C/min, then to 275°C at 10°C/min until 30 min total time. The flow-rate was 1.2 ml/min constant flow. Electron impact ionization (50  $\mu\text{a}$  filament current) and 6  $m/z$  ionization window was used in MS–MS of the analytes. The conditions and criteria for confirmation of PCDDs are shown in Table 1.

Table 1  
GC–MS–MS conditions in the confirmatory analysis of PCP and PCDD/Fs

Analyte	Retention time (min)	Parent ion ( $m/z$ )	Major product ions ( $m/z$ )	Excitation storage level ( $m/z$ )	Resonance excitation amplitude (V)
PCP	11.89	266	228, 202	117	1.6
2,3,7,8-TDCF	17.17	306	243, 269	135	2.5
1,2,3,4-TCDD	17.45	322	259, 285	142	1.5
1,2,3,7,8-PeCDF	18.64	340	277, 303	150	2.5
1,2,3,4,7-PeCDD	19.05	356	293, 319	157	1.5
1,2,3,4,7,8-HxCDF	20.38	374	311	165	2.5
1,2,3,4,7,8-HxCDD	20.85	390	327, 353	172	1.5
1,2,3,4,6,7,8-HpCDF	22.62	410	347	181	3.5
1,2,3,4,6,7,8-HpCDD	23.50	426	361, 389	188	3
OCDD	27.18	460	397, 423	203	1.5
OCDF	27.23	444	381	196	3

### 3. Results and discussion

In our initial SFE ball clay studies, solvent and sorbent blanks indicated a high degree of contamination from a number of sources that interfered with the GC–ECD chromatographic analysis of PCDDs; they included: GC nitrogen and helium gases, house nitrogen, glass wool and SFE valve grease. Precautions were then taken to prevent these contaminants from interfering with the PCDD analysis. GC carry-over from PCDD-containing extracts and standards was eliminated by injecting benzene solvent between each sample analysis.

We found the use of 10,000 p.s.i. (680 bar) at 150°C was required for optimal SFE extraction efficiency of PCDDs from ball clay. At temperatures below 80°C, we obtained TCDD recoveries <30%. In ball clay spiked at the 1 ng/g level, we obtained recoveries ( $n=6$ ) of  $71 \pm 12\%$  for 2,3,7,8-TCDD, and  $71 \pm 10\%$  for the more difficult to extract higher homologue, HpCDD. Recoveries for OCDD were >100% because all the clay samples had a large peak present in the same area of the chromatogram as OCDD; this peak was later confirmed as OCDD. However, recovery of  $86 \pm 9\%$  was obtained for OCDF, which has properties similar to OCDD. A GC–ECD chromatogram from a ball clay SFE extract is shown in Fig. 1. Note the peak at 33.6 min, the same retention time as the OCDD standard, and the very large peak at 12.1 min that corresponded to PCP. Both these and other PCDD peaks that included HpCDD, two congeners of HxCDD and PeCDD, and possible TCDD were also confirmed by GC–MS–MS in the same sample extract. There was no evidence of PCDFs, which are typically found with PCDDs in foods. However, we were concerned about possible artifact formation in ball clay during SFE because high levels of both PCP and PCDDs were present, especially OCDD, estimated at 100 ng/g. This PCDD is often a major contributor to the total TEQ of the food samples [17].

Although a limited number of ball clay samples were analyzed from the Kentucky and Tennessee mines, OCDD and PeCDD were reported as the most abundant congeners [9]. We analyzed ball clays from three different mine sources from this region both before and after addition of 50  $\mu\text{g/g}$  PCP. An GC–ECD chromatogram from one ball clay sample

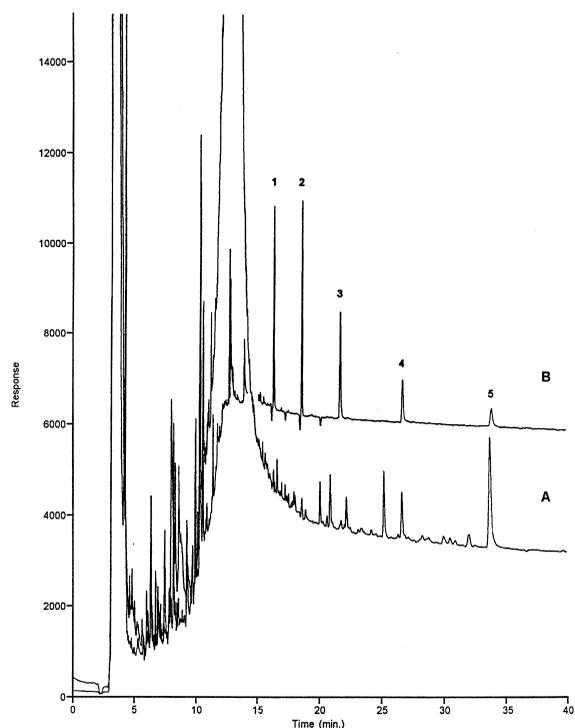


Fig. 1. GC–ECD chromatograms of: (A) SFE extract of ball clay; (B) PCDD standards (peak number): (1) 2,3,7,8-tetrachloro-(TCDD), (2) 1,2,3,7,8-pentachloro-(PeCDD), (3) 1,2,3,4,7,8-hexachloro-(HxCDD), (4) 1,2,3,4,6,7,8-heptachloro-(HpCDD) and (5) octachloro-dibenzo-*p*-dioxins (OCDD).

extracted by SFE is shown in Fig. 2, and the estimated values listed in Table 2 are 17 ng/g OCDD before PCP addition and 27 ng/g afterwards. The table also shows an apparent increase in OCDD for two of the three ball clay samples tested.

The question also arises whether soxhlet extraction may also cause artifactual formation, since extractions take place at the boiling point of the extracting aromatic hydrocarbon solvent. The temperature of the extraction cavity may be higher because of heat transfer from the heating mantle-distillation flask. The long duration of typical soxhlet extractions and the type of sample matrix may also favor PCDD formation. Therefore, we carried out similar studies by soxhlet extraction, and the results compared. In each of the three ball clay samples, an increase in OCDD was noted, but the results from ball clay sample 3 were significantly higher than samples 1 and 2, in the same range as the SFE findings. This

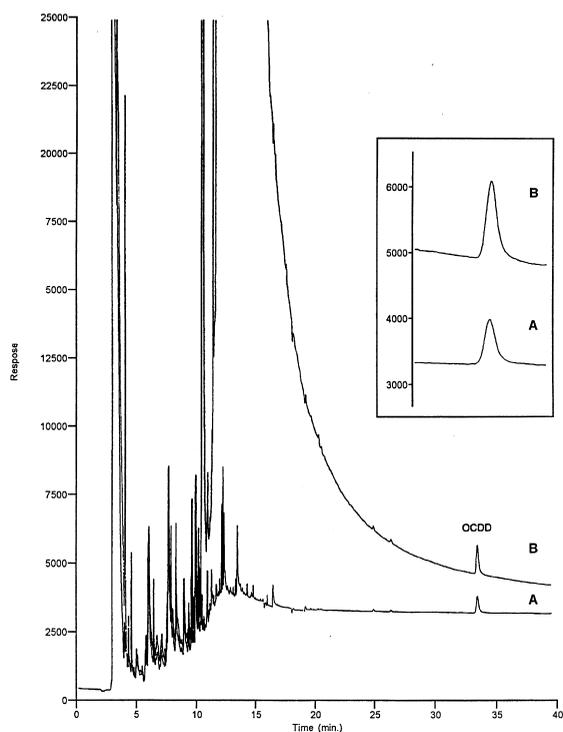


Fig. 2. GC–ECD chromatograms from SFE ball clay extracts: (A) before PCP; (B) after PCP addition.

specific ball clay had the highest published carbon content of the three ball clays tested. Its gray color is attributed to finely divided carbonaceous material

Table 2  
Formation of OCDD from PCP fortified ball clay

	Octachlorodibenzo- <i>p</i> -dioxin, ng/g			
	SFE		Soxhlet	
	–PCP	+PCP <sup>a</sup>	–PCP	+PCP <sup>a</sup>
<i>Ball clay</i>				
1	19	31	10	17
2	17	27	9	16
3	111	98	61	81
<i>Kaolin</i>				
1	0.4	0.6	0.1	0.2
2 <sup>b</sup>	0.2	0.4	0.3	1.0
Bentonite	0.1	0.6	n.d.	n.d.

<sup>a</sup> 50 µg/g.

<sup>b</sup> Clay–carbon (70/30; w/w).

*n* = 2; n.d. = none detected.

derived from fossilized leaves, twigs and other plant debris.

Kaolin was also tested since it comprises 70% or more of ball clay. An increase in OCDD was observed in the SFE extract, but none in the soxhlet. One explanation may be the possible role of the organic material in ball clay that gives it the desirable properties for use in making ceramic products, or that it contains a slightly different mineral content. To simulate the organic content, 30% carbon was added to kaolin to test its effect. Unexpectedly, SFE only gave a slight change in OCDD after PCP addition; much more was obtained by soxhlet extraction as noted in Table 2.

Bentonite, another hydrated aluminum silicate clay, but of volcanic or hydrothermal origin and very little organic matter, was also tested. There was no evidence that this clay favored OCDD formation from PCP. The inability to effectively extract (<30%) OCDD from either kaolin or bentonite by SFE made it difficult to assess the extent of artifactual formation in these clays. However, these findings suggest that the organic material in ball clay may have a positive role in assisting the extraction of these compounds.

#### 4. Conclusions

Formation of OCDD from PCP spiked ball clays by SFE appears to be more complicated than just having the proper temperature and pressure conditions and a large surface area available. In general, more coextractives are obtained by soxhlet than SFE, suggesting SFE has greater selectivity in extraction characteristics. However, our findings suggest that artifactual OCDD can occur in this sample matrix under the SFE conditions we employed, but may be less pronounced under benzene soxhlet extraction conditions. Analysts who routinely measure PCDDs at the low pg/g level need to take precautions against potential contamination and artifactual formation.

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